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Comparative mapping of BTA15 and HSA11 including a region containing a QTL for meat tenderness

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Abstract. The starting point of the present study was the reported identification of a chromosomal region on bovine Chromosome (Chr) 15 (BTA15) carrying loci affecting meat tenderness. A comparative linkage map of BTA15 and human Chr 11 (HSA11) was constructed to identify potential positional candidate genes and to provide a resource of genetic markers to support marker-assisted selection (MAS). Relative rearrangements between the bovine and human genomes for these chromosomes are the most complex observed in comparative mapping between the two species, with nine alternating blocks of conserved synteny between HSA11 and bovine Chrs 15 and 29. The results of this study were the addition of nine genes to the HSA11/BTA15 comparative linkage map, and development of five microsatellite markers within the quantitative trait locus (QTL) interval. One gene with known effects on muscle development (MYOD1) was mapped to the interval. A second gene (CALCA) involved in regulation of calcium levels, a key factor in postmortem tenderization, also mapped within the interval. Refinement of the comparative map and QTL position will reduce the interval on the human transcription map to be scanned in search of candidates, reducing the effort and resources required to identify the allelic variation responsible for the genetic effect.

Introduction

A major goal of livestock genomic research is to understand the basis of the genetic contribution to variation in production traits. However, identification of genes and DNA sequence differences that contribute to relatively minor (but still of substantial economic importance) variation in phenotype is a daunting task. The most promising avenue is the application of comparative mapping, making use of the wealth of knowledge and resources produced from the human genome project and biomedical research community. This approach requires construction of detailed comparative maps, to identify blocks of conserved synteny between species, define the boundaries of these regions, and reveal changes in gene order within these blocks. Some areas of the bovine genome show high degrees of conserved synteny with the human genome, as well as conserved gene order within these areas, while other regions appear to bear a less straightforward relationship (Band et al. 2000). It appears that the most complicated set of relative rearrangements involves genes found on HSA11, which appears to have alternating blocks of conserved synteny with BTA15 and BTA29.

Recent construction of microsatellite-based genetic linkage maps for cattle has made it possible to identify chromosomal regions carrying loci that affect meat quality, reproductive efficiency, and growth (Stone et al. 1999; Casas et al. 1998; Keele et al. 1999). These mapping studies typically make use of diverse germplasm, such as interbreed crosses, which provide strong phenotypic contrasts and increase the level of marker informativeness. Meat tenderness is one important carcass quality trait to the beef industry (NCBA 1998) for which a chromosomal region affecting phenotype has been previously described (Keele et al. 1999). In summary, Warner-Bratzler shear force measurements were obtained on days 2 and 14 post-mortem for 294 progeny from a mating of a Brahman × Hereford bull to *Bos taurus* cows. A genome scan identified a QTL on BTA15 with a peak at 28 cM from the most centromeric marker (MGTG13B) on the U.S. Meat Animal Research Center (MARC) linkage map (Kappes et al. 1997), with a 95% confidence interval spanning 23 cM (between positions 17 and 40 cM on the map).

The goals of the present study were to generate additional markers for use in marker-assisted selection and refinement of the QTL interval, and to identify positional candidate genes by using comparative mapping to the gene-rich human map. Human Chr 11 shows alternating blocks of conserved synteny with BTA15 and BTA29 (Chowdhary et al. 1996; Band et al. 2000; Amarante et al. 2000), but the low resolution with which these blocks have been defined complicates the selection of positional candidate genes. In order to improve this resolution and provide potential candidate genes, we added nine genes to the bovine linkage map, three of which fell within the QTL interval.

Materials and methods

PCR. Primer sets for nine genes were designed from sequence data available in GenBank or obtained from the literature (Table 1). Primer sets for microsatellite screening of the RH panel were obtained from the MARC website (http://www.marc.usda.gov/). Standard polymerase chain reactions (PCR) included 50 ng DNA, 100 μM each of the four nucleotides, 0.1% Triton X-100, 50 mM KCl, 10 mM Tris-HCl pH 9.0, 1.5 mM $MgCl_2$, 0.5 μM each of forward and reverse primers, and ddH_2O to a total reaction volume of 10 μl. Reactions were conducted in a PTC-200 DNA Engine (MJ Research, Incline Village, NV). Thermocycling parameters were 95°C for 10 min, 35 cycles of 95°C for 30 s, 55–63°C for 30 s, and 72°C for 30 s, with a final extension cycle of 72°C for 5 min. PCR products were electrophoresed on 2% agarose gels and visualized with ethidium bromide.

Sequencing. Reactions were performed as recommended (Applied Biosystems, Foster City, CA). Single nucleotide polymorphisms (SNPs) were identified by visual inspection of the chromatograms or by alignment with the programs phrap, polyphred, and consed. Sequences were compared with the GenBank database via BLASTN search (Altschul et al. 1990) to verify that amplicons were specific to the target genes. PCR products were generated from within each gene for each of the four F1 bulls in the MARC reference families (Bishop et al. 1994) and sequenced for SNP discovery.

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Table 1. Primer information.

Gene (symbol)	Primer sequences	Accession for primer design	Accession of product	SNP position[a]	Alleles[b]
Acid sphingomelinase (ASM)	F tgtggaaaggtatgagaacacc R tccgtcagattcatgatgtagg	AW307984	AF325550	169	A/G
Adrenomedullin (ADM)	Jiang et al. 1998	AJ001613	Same[c]		
Amyloid beta precursor protein-binding B1 (APBB1)	F attccttttggaaccccaac R ggtccttcagccccaaat	AW326116	AF325551	222	T/G
Brain derived neurotrophic factor (BDNF)	Jiang et al. 1998	X97914	Same[c]		
Calcitonin (CALCA)	F gagacagaggactccaggtaag R agtggaaggagttcagttggc	AJ271090	AF297204		
Collagenase (MMP1)[d]	F atgaagcagcccagatgtg R cttggaaattgctggtccaccat	AF134714	AF297203		
Glucose-6 phosphatase transporter (G6PT1)	F aggagatccctctggacaaa R aaacttgctgatggcgtagg	AW447228	AF325552	356	T/C
Myogenic determination factor 1 (MYOD1)	Defined in AF297205	AF093675	AF297205		
Nuclear mitotic apparatus protein	F aagcgggtttcctcagagac R gttgggtgaggctttggag	AW355010	AF325553	81	T/C

[a] The nucleotide position in the sequence shown under "accession of product" at which the genotyped polymorphism is located.
[b] The alternative alleles observed in the MARC mapping; parent bulls are indicated.
[c] Sequence of PCR product is identical to that of the sequence used to design primers.
[d] Previously reported primers for MMP1 (Jiang et al. 1998) were aligned to bovine GenBank sequences, and base pair changes were made to increase similarity to the bovine gene.

Radiation hybrids. Primer sets for seven microsatellites (MARC database; http://www.marc.usda.gov/) that span the BTA15 QTL interval were used to screen a bovine RH panel (Womack et al. 1997). Ninety hybrids were screened for the presence of the seven microsatellites and six genes whose PCR product showed a bovine specific fragment. Each marker was run on the panel twice, and each hybrid was scored as +, −, or? for each marker. Data were analyzed by using RHMAP3.0 (Lange et al. 1995). A primer pair that amplifies bovine CAPN1 and has been mapped to BTA29 (Smith et al. 2000), was screened on the RH panel, and the resulting data were used in the two-point analysis. HSA11 genes not showing co-retention with markers on BTA15 would be expected to show co-retention with markers on BTA29 such as CAPN1 (Solinas-Toldo et al. 1995; Chowdhary et al. 1996; Band et al. 2000).

YAC screening. YAC superpools and secondary pools from the MARC YAC library were screened with microsatellites in the QTL region (JAB1 and BMS1782) or gene-specific primers (ADM, BDNF, and CALCA). Once individual YAC clones were identified, they were subcloned (Sonstegard et al. 1997) and probed for microsatellites by using a $(GT)_{11}$ probe. Alternatively, SINE-PCR (Lenstra et al. 1993) products from reactions using individual YAC clone DNA for template were cloned and probed for microsatellites.

BAC screening. Gene-specific probes for hybridization were constructed from gel-purified PCR products and radioactively labeled by using random-primed labeling (Megaprime DNA Labeling System, Amersham RPN 1605) and used to probe a bovine BAC library (RPCI-42) obtained from Roswell Park Cancer Institute (Warren et al. 2000). Cocktails of multiple probes were hybridized to filters containing over 18,000 clones (in duplicate) each. Positive clones were then picked and grown in 96-well plates. Sorting positive clones by gene was done by PCR or by secondary hybridizations to filters stamped from 96-well plates followed by PCR verification. Individual clones were regrown in a high nutrient broth for DNA extraction by alkaline lysis (Kirschner and Stratakis 1999), as suggested by the producer (Roswell Park Cancer Institute). BAC DNA was then subcloned and probed for microsatellites (Table 2).

Linkage analyses. Marker genotypes were generated for the parents of the MARC reference families (Bishop et al. 1994). Families including parents that were determined to be heterozygous for the markers were then genotyped and the data merged with those of the 1500 markers in the MARC database (Keele et al. 1994; http://www.marc.usda.gov/cattle). CriMap Version 2.4 (Green et al. 1990) was used to perform two-point and multi-point analyses as described (Kappes et al. 1997).

Results

Nine genes (ADM, APBB1, ASM, BDNF, CALCA, G6PT1, MMP1, MYOD1, and NUMA1; see Table 1 for definitions) mapping to HSA11 were targeted as possible comparative candidate loci for the QTL or as markers to define breakpoints in conserved synteny between the two cattle chromosomes and HSA11. Primers developed for each gene were tested on bovine genomic DNA, and the product was sequenced to verify that the correct gene had been targeted. Owing to the "shuffling" of genes during evolution, resulting in some HSA11 orthologs lying on BTA29, a preliminary screen for synteny of six genes with microsatellites chosen from BTA15 was performed with a bovine radiation hybrid (RH) panel. Primers designed for APBB1, ASM, and G6PT1 were not suitable for this analysis owing to production of amplicons from the rodent control DNA. The average retention frequency for all markers was 13.1%. Pairwise analysis (Rexroad, unpublished) revealed that ADM, ASM, BDNF, CALCA, NUMA1, and MYOD1 all had significant (LOD > 3.0) co-retention frequencies with the seven microsatellites typed on the RH panel. This is consistent with a recent report of typing on the same RH panel with different primers for ADM (Band et al. 2000). The amplicon for CAPN1, which maps to BTA29, did not show significant co-retention with these markers. MMP1 showed weak co-retention in the RH panel with markers from BTA15 (LOD > 2.0), but showed no significant co-retention with CAPN1. This is consistent with linkage data (below) that suggest that MMP1 maps to BTA15 but not in close proximity to the targeted QTL interval, and with recently published results by using the same RH panel that indicates a map position at the centromeric end of the chromosome (Amarante et al. 2000). Multipoint analysis of the RH data resulted in a large number of possible orders, none of which had significant confidence compared with the alternative orders (data not shown).

The gene-specific PCR products generated from genomic DNA of the four F_1 bulls of the MARC mapping population (Bishop et al. 1994) were sequenced to identify single nucleotide polymorphisms (SNPs). This procedure identified SNPs in G6PT1, APBB1, NUMA1, and ASM, which were used to place these four genes on the bovine linkage map (Fig. 1). Details of the SNPs are given in Table 1. None of the other five genes had SNPs in the amplified fragments from these bulls. To map these genes, large insert clones were obtained by screening bovine BAC and YAC libraries with gene-specific primers. To provide additional markers in the QTL interval, the bovine YAC library was also screened with microsatellite markers previously mapped to this region of BTA15. Additional microsatellite markers were then developed from these clones by subcloning, screening, and sequencing. In total, 10 microsatellites were added to the current BTA15 linkage map (http://www.marc.usda.gov), five of which map to the QTL

Table 2. Amplification and mapping information for microsatellite markers. GenBank accession numbers are given for each marker as well as the clone identification number for the BAC or YAC clone from which the marker was derived (primer sequences used for amplification are defined in the GenBank entries). The number of alleles observed and the number of informative meioses in the MARC mapping population, the size range of the observed alleles in bp, and the estimated position of the marker in the BTA15 linkage group are indicated.

Marker name	Associated locus[a]	BAC/YAC ID	Accession number	No. of alleles	Allele sizes	No. of informative meiosis	cM
BY1502	(JAB1)	YAC 151 C 10	AF355778	6	124–140	338	28.2
BY1503	(BMS1782)	YAC 304 B 7	AF355777	2	148–150	52	36.8
BY1520	BDNF	YAC 311 E 10	AF297205	7	221–239	289	72.3
BB1525	ADM	BAC 303 P 21	AF297206	3	202–216	98	49.0
BB1526	ADM	BAC 303 P 21	AF297207	6	169–179	230	46.8
BB1528	MMP1	BAC 382 L 10	AF297208	7	321–343	227	0
BB1529	CALCA	BAC 352 N 10	AF297209	5	96–116	174	39.2
BB1533	MYOD1	BAC 196 M 14	AF297210	2	129–131		ND[b]
BB1538	MYOD1	BAC 196 M 14	AF297211	3	147–149	102	40
BB1539	MYOD1	BAC 196 M 14	AF297212	6	217–231	260	37.1

[a] Gene symbols are given for gene-related markers; loci symbols in parentheses represent previously mapped non-gene markers.
[b] BB1533 is known to contain a null allele; difficulties in genotyping did not allow it to be positioned on the map by using the MARC reference families.

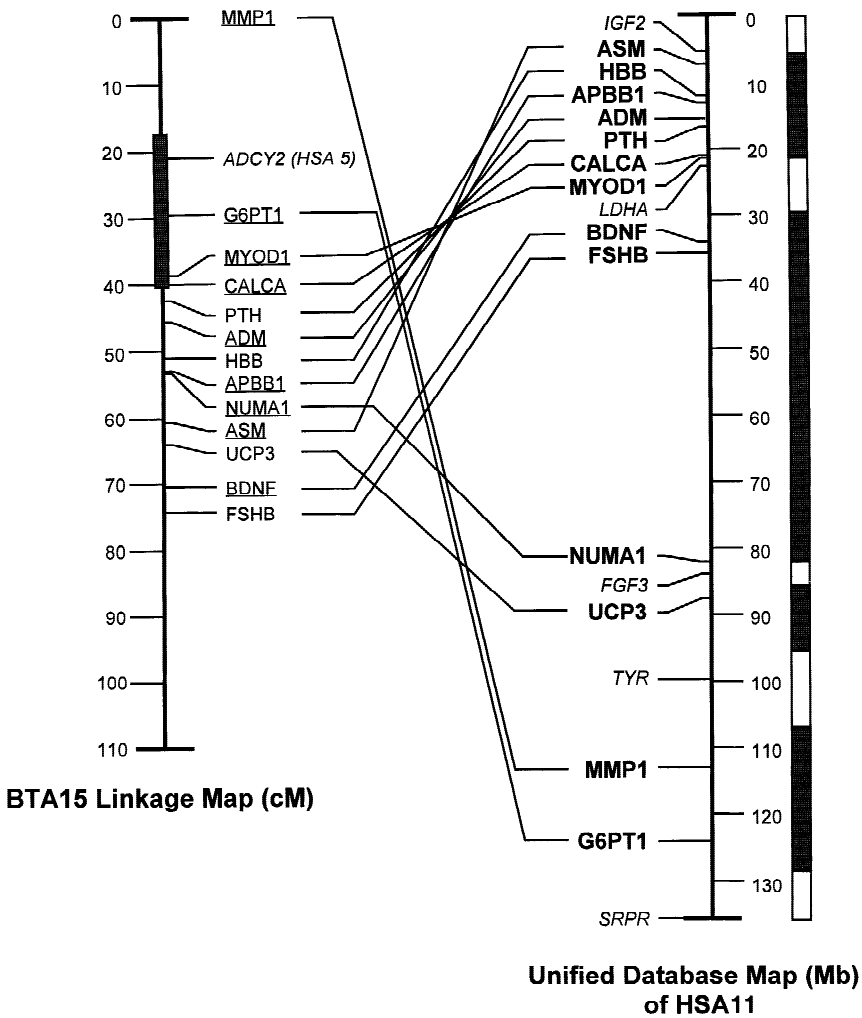

Fig. 1. A comparative map including a rendering of the Unified Database HSA11 physical map (http://bioinformatics.weizmann.ac.il/udb/) and genes on the BTA15 linkage map. Genes in **italics** on HSA11 map to BTA29, those in **bold** map to BTA15. The bar to the right of the HSA11 map indicates the portions apparently having conserved synteny with BTA15 (gray rectangles) or BTA29 (open rectangles), to emphasize the nine alternating blocks in the comparative maps. On the BTA15 map shown, the fact that ADCY2 maps to HSA5 is indicated. The gray bar on the BTA15 linkage map indicates the 95% confidence interval for the meat tenderness QTL. Genes that were added to the BTA15 linkage map in the current study are underlined.

interval. Using large insert clones resulted in the addition of five genes to the comparative map of BTA15 (Fig. 1).

Two of the seven microsatellites (BY1502, BY1503) obtained from subcloning YACs that screened positive for JAB1 or BMS1782 mapped to BTA15. The other five microsatellites mapped to other chromosomes (Rexroad et al. 2000), apparently as a result of chimerism in the YAC library. SINE-PCR has proven a valuable tool for the discovery of microsatellite markers in YACs, but the repetitive elements found near these microsatellites complicate primer design. The same is true for attempts to design SNP markers from SINE-PCR products. Difficulties with chimerism and YAC DNA recovery caused us to shift our gene mapping and marker discovery efforts to BACs, using YACs primarily for contig construction. BACs have the potential for recovery of greater yields of low-copy plasmid DNA and are known to display lower rates of chimerism than YACs.

Discussion

One approach to discovering specific allelic variation responsible for genetic effects is fine mapping, with the goal of identifying candidate genes in the chromosomal segment containing the QTL and producing additional genetic markers to refine the QTL peak. Comparative mapping between bovine and human genomes is the most efficient way to identify positional candidate genes, as it makes use of the wealth of data available from the human map and biomedical community. By using this approach, new markers can be identified in the QTL region, and potential positional candidate genes can be identified and evaluated.

Fine mapping of the meat tenderness QTL on BTA15 resulted in the development of 10 microsatellite markers and the mapping of nine genes to this linkage group. The addition of BB1528, 6 cM from the previous most centromeric marker of the linkage map, extends the centromeric boundary of the bovine linkage map for this chromosome. Difficulties in genotyping BB1533 prevented a precise positioning on the bovine linkage map; however, the pairwise analysis showed a recombination fraction of .07 at LOD 6.99 with BB1538, which maps in the QTL interval. The mapping of five new microsatellite markers within the QTL interval on a reference family map provides additional resources to refine the QTL position.

Use of the pairwise RH data proved to be useful as it yielded confidence that the chosen loci were in close proximity to the QTL. Although the resolution of the RH panel was relatively poor for this chromosome such that genes could not be ordered with confidence, it provided a rapid way to verify that genes would map to BTA15 rather than BTA29. Owing in part to low retention frequencies and a relatively low rate of breakage in the RH panel used, the RH data alone were insufficient to conclude which genes map in the QTL interval. Gene-specific products showing physical linkage to markers in the QTL interval were then used as starting points for genetic marker development, to more accurately map them relative to the interval.

Relative chromosomal rearrangements between the bovine and human genomes with respect to genes found on HSA11 are more complex than any others observed on the comparative map of these species. Placement of these nine genes on the linkage map contributes to a comparative map revealing nine blocks of conserved synteny between HSA11, BTA15, and BTA29 (Fig. 1). Although this increase in information on the comparative map aids the selection of comparative candidate loci, the number of gene order changes observed so far suggests that no gene may be confidently assigned by association with a mapped gene, but must be individually mapped to insure correct assignment.

Adenylate cyclase 2 (ADCY2) maps to BTA15 and HSA5 (Chowdhary et al. 1996; Band et al. 2000) and was previously the most centromeric gene on the comparative map. BB1528 is a microsatellite associated with MMP1 that maps to the centromeric end of BTA15 and increases the linkage group by 6 cM. MMP1 maps to HSA11, revealing a region on BTA15 homologous to HSA5 flanked by regions homologous to HSA11.

Genes that map to a position within the QTL interval can be considered as candidate loci, possibly containing the sequence variation responsible for the genetic effect on the trait. Although the glucose-6 phosphatase transporter G6PT1 can not be ruled out as a candidate because of possible effects on glycolytic processes and potential effects on muscle pH, a direct role of allelic variation at this locus on meat tenderness does not appear straightforward. Of the genes included in this study, MYOD1 and CALCA have the highest potential as positional candidate genes for this meat tenderness locus. CALCA is involved in the regulation of intracellular calcium (Zink-Lorenz et al. 1996). Calcium concentration is known to play a role in meat tenderness by influencing the calpains, a family of calcium-dependent proteases (Wheeler et al. 1997). It is conceivable that any genetic variation in the CALCA gene affecting how the protein functions to regulate intracellular calcium could also affect meat tenderness. MYOD1 is a transcription factor expressed in skeletal muscle myogenesis and regeneration (Atchley et al. 1994; Fuchtbauer et al. 1992). Although a direct mechanism by which allelic variation in MYOD1 during early muscle development might affect postmortem tenderization is not immediately obvious, it is possible that genetic variation in this gene changing its ability to influence the expression of structural components of muscle could affect meat tenderness. Future studies to assess the possible variation and impact of these candidate genes on meat tenderness may be able to address possible mechanisms.

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